IMMUNE REACTIONS IN SCARLET FEVER, II*

ANTIGENIC PROPERTIES OF BACTERIA FOUND IN SCARLATINA

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In a previous report[1] the results of a number of complement-fixation tests in scarlet fever were given. In these tests the blood serum, spleen, and lymph glands, used as antigen, failed to indicate the presence of a specific virus. With mucus from scarlatinal angina as antigen, a weakly positive reaction was obtained in one instance. On the supposition that the antigenic property of the mucus was due to micro-organisms capable of growths, cultures were made of the mucus and tested as to antigenic properties by means of complement-fixation and intracutaneous reactions.

COMPLEMENT-FIXATION

Antigens 1, 2, and 3.—On account of the evidence that scarlatina is at times transmitted by means of milk, cultures were made as follows:

A flask of sterile whey titrated to 0.5% acidity and a flask of sterile milk were inoculated with mucus from the throat of a scarlatinal patient who had been ill 2 days. After 4 days' incubation at 36 C. the milk had become coagulated. The whey was removed from it by passage through a Berkefeld filter, and the filtered whey added to the other whey, which contained a heavy growth of bacteria. The mixture was then heated at 60 C. for 1 hour.

This antigen was then employed in complement-fixation tests on the sera from 20 cases of scarlatina. A sheep-erythrocyte antisheep-rabbit-serum system was used with guinea-pig serum as complement; 2 units of amboceptor were used. By preliminary tests it was found that diluted antigen (1:1) in quantities of 0.05 c.c., 0.1 c.c., 0.15 c.c., and 0.2 c.c., gave the most satisfactory results; 0.2 c.c. of antigen usually gave slight inhibition. The scarlatinal serum was used at first in quantities of 0.025 c.c., 0.05 c.c., and 0.1 c.c., both active and inactivated. In later tests, however, 0.05 c.c. of the active serum only was used. By testing the hemolytic power of this serum corrections could be made so that the total complement and amboceptor were fairly constant in each test.

A control antigen (2) was made from mucus from the throat of a case of Ludwig's angina, the same method being employed as for the scarlatinal antigen. The scarlatinal sera were controlled by sera from cases giving no

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[1] Jour. Infect. Dis., 1916, 19, p. 175

histories of scarlatina. These controls were uniformly negative. A third antigen was made by the method employed in the case of Antigen 1, mucus from the throat of another scarlatinal patient being used.

The results of these tests are shown in Table 1. Reactions are designated as + when slight inhibition was obtained in tubes containing one-fourth the quantity of antigen which alone showed inhibition. A well-marked inhibition is designated as ++, and complete inhibition as +++.

TABLE 1

RESULTS OF COMPLEMENT-FIXATION TESTS WITH ANTIGENS 1, 2, AND 3

Case and Description			Result of Test		
Number	Day of Disease	Temperature	Antigen 1 Scarlatinal Throat	Antigen 3 Scarlatinal Throat	Antigen 2 Ludwig's Angina
38	2	101	++	+	—
50	2	Normal	±	++	—
45	3	104.5	—	..	—
36	3	Normal	—	..	—
30	4	100.5	+++	..	++
33	4	Normal	+++	—	—
39	4	Normal	++	+	—
40	4	Normal	++	..	..
42	4	Normal	—	..	..
31	5	Normal	+++	..	..
41	7	Normal	+	..	..
35	11	Normal	++	..	..
34	12	Normal	+++	..	..
47	15	Normal	—	..	..
32	18	Normal	+++	..	..
47	22	Normal	—	..	..
44	23	Normal	—	..	—
37	24	Normal	++	..	—
36	27	Normal	++	..	..
49	31	Normal	±	..	++
43	32	Normal	—	..	+++
48	34	Normal	±	..	++

It will be seen from this table that with Antigen 1 fixation occurred in 12 of the 22 cases (54.6%). No fixation was obtained in 7 cases (31.8%), and in 3 cases the result was questionable (13.6%). With Antigen 3 a higher percentage of positive results was obtained, but the number of cases tested was small. Wassermann tests were negative in all the sera tried. With the antigen from Ludwig's angina, positive reactions occurred in one-third of the cases tried and negative reactions in two-thirds. The cultures from scarlatinal throats, then, showed the presence of an antigen which reacted with scarlatinal serum to fix complement in more than one-half the cases tested, whereas control antigen made from Ludwig's angina gave positive reactions in over one-third of the cases tested.

Antigens 4, 5, and 6.—With the object of finding the incubation period of the antigen present in the cultures of scarlatinal mucus the following experiment was made.

A flask of ordinary broth was inoculated with mucus from a scarlatinal throat. A part of this inoculated broth was at once heated to 60 C. for 1 hour and stored at 5 C. The remainder was incubated at 36 C. for 24 hours and again a portion removed and heated to 60 C. for 1 hour and stored at 5 C. The original flask was re-incubated for another 24 hours and then heated to 60 C. for 1 hour. In this way 3 antigens were obtained; one not incubated, one incubated 24 hours, and a third incubated 48 hours.

The three antigens were then examined by means of complement-fixation to see whether there was any increase of antigenic strength with incubation. These complement-fixation tests were carried out in a way similar to those described for Antigens 1, 2, and 3, 0.05 c.c. of complement and 2 units of amboceptor being used. The inactivated (at 56 C. for one-half hour) serum of a convalescent scarlatinal patient was used in the constant quantity of 0.1 c.c. in all the tests.

The result is shown in Table 2. The slight fixation obtained in the original broth-mucus mixture was increased in 24 hours to a strong fixation. In 48 hours the antigen alone began to inhibit more, while the fixation with scarlatinal serum was not further increased. The best antigen, therefore, was that of the 24-hour incubation.

TABLE 2

ANTIGENIC STRENGTH OF BROTH CULTURES OF ANGINAL MUCUS AT THREE STAGES OF INCUBATION

Stage of Incubation		Quantities of Antigen Used					
		0.05 c.c.	0.1 c.c.	0.2 c.c.	0.3 c.c.	0.5 c.c.	1 c.c.
Before incubation, Antigen 4	Antigen alone.....	—	—	—	—	—	+
	With scarlatinal serum	—	—	—	+	..	..
After 24 hours, Antigen 5....	Antigen alone.....	—	—	—	—	—	+
	With scarlatinal serum	—	—	++	+++	..	..
After 48 hours, Antigen 6....	Antigen alone.....	—	—	—	±	±	+
	With scarlatinal serum	—	—	++	+++	..	..

Antigen 7.—Examinations of Antigens 1 to 6 showed that the predominant growth in 24 hours was that of streptococci. In order to compare the antigenic property of streptococci with that of the mixed cultures of the preceding experiment, a hemolytic streptococcus was isolated from scarlatinal angina and a broth antigen prepared as in the case of Antigens 4, 5, and 6.

With the same technic as before tests were carried out in 9 cases of scarlatina. Of these 9 cases, the sera of 8 (88%) showed fixation of complement, which in 4 instances was complete. These results would indicate that no antigenic property need be assumed in any of the antigens prepared from scarlatinal throats other than that of the streptococci present.

TABLE 3

COMPARISON OF THE ANTIGENIC STRENGTHS OF CULTURES OF THE VARIOUS ORGANISMS IN ANGINAL MUCUS

Serum	Antigen										
	7	8	9	10	11	12	13	14	15	16	17
18	..	..	..	..	..	..	..	..	..	..	..
20	+	..	+	+	..	..	..	..	+	..	..
21	..	..	..	..	+++	+++	+	..	..	..	..
22	..	++	..	..	..	..	..	++	..	++	++
23	++	..	+	+	..	..	..	..	..	..	..
24	+++	..	—	+	..	..	..	..	..	..	..
25	—	..	—	—	..	..	..	..	..	..	..
26	++	..	—	—	..	..	..	..	..	..	..
27	+	..	—	—	..	..	..	..	..	..	..
28	+++	..	—	+	..	..	..	..	..	..	..
29	+++	..	—	+	..	..	..	..	..	..	..
30	+++	..	—	++	..	..	..	..	..	..	..
31	..	..	..	..	..	..	..	..	..	..	..
32	..	..	..	..	..	..	..	..	..	..	..
33	..	..	..	..	..	..	..	..	..	..	..
34	..	..	..	..	..	..	..	..	..	..	..
35	..	..	..	..	..	..	..	..	..	..	..
36	..	..	..	..	..	..	..	..	..	..	..
37	..	..	..	..	..	..	..	..	..	..	..
38	..	..	..	..	..	..	..	..	..	..	..
39	..	..	..	..	..	..	..	..	..	..	..
40	..	..	..	..	..	..	..	..	..	..	..
41	..	..	..	..	..	..	..	..	..	..	..
42	..	..	..	..	..	..	..	..	..	..	..

* Control sera from convalescent diphtheria cases +++ and +.
† Control sera from convalescent diphtheria cases +++ and ±.

Other Antigens.—In order to compare the antigenic property of streptococci with that of other organisms from scarlatinal anginas, a number of organisms were isolated in pure culture and antigens prepared from them. When possible these antigens were prepared in the same manner as the preceding ones, but in some cases other cultural methods were necessary. A brief description of these organisms follows:

8. Streptococcus similar to that of Antigen 7.

9. Bacillus: Slender, tapered, with tendency to slight curve; gram-negative; aerobic. Good growth on ordinary media, mannite, milk, lactose, and dextrose; unchanged at end of 48 hours. Slight green discoloration produced in blood agar. Growth formed a grayish-yellow, very moist film.

10. Micrococcus tetragenus.

11. Bacillus: Large; spore-forming; gram-positive; aerobic. Good growth on ordinary media; opaque cream-colored moist growth on blood agar.

12. Bacillus: Small; gram-positive; aerobic. Opaque white growth on blood agar; color of blood changed to green.

13. Bacillus: Small; gram-positive; ends tapered; tendency to slight curve, especially where it occurs in pairs; aerobic. White opaque moist film on blood agar; slightly hemolytic.

14. Diplobacillus: Gram-positive; aerobic; hemolytic. Convex brownish opaque colonies on blood agar.

TABLE 3—*Continued*

COMPARISON OF THE ANTIGENIC STRENGTHS OF CULTURES OF THE VARIOUS ORGANISMS IN ANGINAL MUCUS

Antigen											
18	19	20	21	22	23*	24	25	26	27	28†	29
..	..	..	..	..	..	..	—	..	..	..	..
..	..	..	..	..	..	..	..	..	..	..	..
..	..	..	..	..	..	..	..	..	..	..	..
..	..	..	..	..	..	..	..	..	..	..	..
—	+	—	..	..	..	..	..	—	..	..	..
—	—	—	+	—	..	..	..	—	..	..	..
—	—	—	—	—	..	..	..	—	..	..	..
—	+	—	—	+	..	..	..	—	..	..	..
—	—	—	—	..	..	..	..	—	..	..	..
—	—	—	—	—	..	..	..	..	..	..	..
—	—	—	..	—	..	..	..	..	..	..	..
—	—	—	..	—	..	..	..	..	..	..	..
..	..	..	..	..	..	..	..	..	—	..	..
..	..	..	..	..	..	..	..	..	+++	..	..
..	..	..	..	..	..	..	..	..	+++	..	..
..	..	..	..	..	..	..	..	..	±	..	..
..	..	..	..	..	..	—	..	..	..	—	..
..	..	..	..	..	..	—	..	..	..	—	..
..	..	..	..	..	..	—	..	..	..	—	..
..	..	..	..	..	—	..	..	..	..	..	..
..	..	..	..	..	—	..	..	..	..	..	..
..	..	..	..	..	..	..	..	..	..	..	..
..	..	..	..	..	..	..	..	..	..	..	..

15. Bacillus: Small; gram-positive; occurring in chains; anaerobic; hemophilic. Blood unchanged; grayish translucent film on blood agar.

16. Bacillus: Very small; gram-negative; anaerobic. No growth on ordinary media; no change in blood; pinpoint transparent colonies on human-blood agar.

17. Bacillus: Small, with great variation in length; tendency to occur in chains; some of longer forms showed vacuoles; gram-negative; anaerobic. No growth on ordinary media; delicate transparent film on human-blood agar; slight but constant hemolysis. Specimens stained with methylene blue somewhat resembled diphtheria bacillus.

18. Bacillus mucosus: Short; encapsulated; gram-negative; aerobic; mucoid growth; hemolytic. Dextrose, mannite, and lactose acid; milk acid and coagulated.

19. Bacillus: Formed chains; gram-positive; aerobic. Small convex grayish opaque colonies or yellowish opaque raised film; good growth on ordinary media; dextrose, lactose, and mannite acid; milk acid but not coagulated; blood hemolyzed.

20. Bacillus: Spore-forming; gram-positive; aerobic. Formed dry finely wrinkled film on surface of plain agar; hemolytic.

21. Streptobacillus: Gram-positive; anaerobic; hemolytic. Lactose, mannite, and dextrose-litmus agar bleached to canary-yellow color; litmus milk bleached and not coagulated; grayish transparent film on surface of blood agar.

22. Bacillus mucosus similar to No. 18.

23. Coccus: Oval; occurred in pairs; did not form chains; gram-positive; aerobic. Grew on ordinary media; no change in dextrose, lactose, mannite-litmus agar, litmus milk, or blood agar.

24. Streptothrix: Branched thread-like organism; gram-positive; anaerobic. No growth on ordinary media; growth on human-blood agar slow, forming transparent film at end of 48 hours, which at the end of 4 days changed to brownish opaque layer; single colonies became umbilicated.

25. Bacillus: Very small; gram-negative; anaerobic. No growth on ordinary media; blood unchanged.

26. Bacillus: Thread-like; gram-negative; anaerobic. No growth on ordinary media; growth on human-blood agar scant, forming transparent film without change in the blood.

27. Bacillus: Varying from short coccoid form to thread-like structure; encapsulated; aerobic; characterized by irregular unstained areas and mucoid nature of growth; gram-negative.

28. Bacillus: Very small; gram ± at end of 24 hours; aerobic. Numerous minute grayish translucent colonies on blood agar; no effect on blood.

29. Diphtheroid: Gram-positive; aerobic; pleomorphic. Yellowish-gray raised growth; good growth on ordinary media.

It is evident from Table 3 that the sera tested may be divided into 2 types: (1) sera which tend to fix complement with a number of antigens, such as Sera 20 and 21; and (2) sera which show little tendency to fix complement with any antigen, such as Sera 25 and 27.

Livierato,[2] in a study of the antibodies developed during the acute exanthemata, divided them into specific and associated antibodies. For instances, in 10 cases of erysipelas, 2 cases showed antibodies for streptococcus alone, 2 for staphylococcus, 1 for pneumococcus, 1 for streptococcus and staphylococcus, 2 for streptococcus and gonococcus, 1 for streptococcus and the typhoid bacillus, 1 for streptococcus, staphylococcus, and pneumococcus. In measles, peliosus rheumatica, variola, and varicella, similar associated antibodies were found. Livierato failed to find associated antibodies in scarlet fever. This may have been due to the organisms used as antigens. The fixation of complement with a number of antigens may indicate a development of nonspecific immune bodies or the development of specific antibodies to a number of organisms. The only organism tested which appears to produce immune bodies with any degree of constancy in scarlatina is the streptococcus. This is in accord with the results of Tunnicliff,[3]

[2] Gazz. d. osp. Milano, 1907, 28, p. 835.

[3] Jour. Infect. Dis., 1907, 4, p. 304.

Schleissner,[4] and others. Kolmer,[5] however, found fixation of complement with streptococcus antigen in only 11.2% of sera from scarlet-fever convalescents.

CUTANEOUS TESTS

Cutaneous tests on convalescent scarlet-fever patients were carried out with antigens made as for the complement-fixation tests. As controls, diphtheria patients were used who gave no history of scarlet fever. The technic used was similar to that of the intracutaneous tuberculin and Schick tests. Preliminary experiments with the von Pirquet scarification technic were unsatisfactory. The antigen was a heat-sterilized culture of mucus from scarlatinal angina.

In 12 scarlet-fever patients there developed at the site of injection areas of reddening and induration which reached a maximum on the day following the injection and then gradually disappeared. The controls showed much less marked reactions.

In order further to investigate this reaction the following study was made. Three antigens were prepared: (1) a milk and whey culture of mucus from scarlatinal angina; (2) a pure culture of a hemolytic streptococcus from a fatal scarlatinal angina; (3) a milk and whey culture from an ordinary follicular tonsillitis due apparently to streptococcal infection. The three antigens were injected intracutaneously on the outer aspect of the upper arm about 4 cm. apart. In this way the reactions following the injection of the three antigens were compared with each other and with control reactions obtained in the same way in diphtheria convalescents. One-tenth cubic centimeter of fluid containing, according to turbidity, about the same quantity of bacteria was used for each injection. Accurate measurements of the areas of reaction which occurred are shown in Table 4. It will be noted that in some instances one diphtheria control served for more than one of the 10 scarlet-fever patients.

The reactions in the scarlatinal convalescents were as follows:

Scarlatinal-Throat Antigen.—An area of reddening of the skin developed in a few hours and reached its height in about 24 hours, when it averaged about 2 cm. in diameter. At the end of the 2nd day the redness began to disappear and was gone by the 3rd or 4th day. The reddening was accompanied by an induration, which developed a little more slowly, but also reached a maximum in about 24 hours,

[4] Wien. klin. Wchnschr., 1909, 22, p. 553.
[5] Arch. Int. Med., 1912, 9, p. 220.

TABLE 4

CUTANEOUS REACTIONS IN SCARLATINA

Case	Disease and Day of Disease	Antigen	Reaction (cm.) 1st Observation		Reaction (cm.) 2nd Observation		Reaction (cm.) 3rd Observation	
			Reddening	Induration	Reddening	Induration	Reddening	Induration
F. O. 12 years	Diphtheria 1 month	Scarlet fever Streptococci Tonsillitis	5 hr. 2	5 hr.	28 hr. 0.8×0.5 1.7×1.8 1.5	28 hr. 0.5 1.5 0.7	50 hr. 0.5×0.6 0.7×0.6 0.2	50 hr. 0.2 0.8
M. R. 12 years	Scarlet-fever convalescence 5 weeks	Scarlet fever Streptococci Tonsillitis	5 hr. 1 3	5 hr. 1 3.5 edema	28 hr. 1.2×1 2.5 7×8	28 hr. 0.5×1.0 1 7×8edema	50-72 hr. 0.7×6 1×1.4 0.5	50-72 hr. 0.7×0.5 0.8×1 1
E. W. 3 years	Scarlet-fever and diphtheria 3 weeks	Scarlet fever Streptococci Tonsillitis	5 hr. 0.5 0.2 1	5 hr. 1 0.5 1	28 hr. 0.3 0.3 1.7	28 hr. 0.5 0.3 1.0	50-74 hr.	50-74 hr. 0.5 0.5
M. P. 9 years	Scarlet fever 3 weeks	Scarlet fever Streptococci Tonsillitis	3 hr. 1	3 hr. 1 edema	5 hr. 1	5 hr. 1 edema	26 hr. 6 1 3	26 hr. 1 0.5
M. K. 8 years	Diphtheria convalescence	Scarlet fever Streptococci Tonsillitis	3 hr. 0.5 0.3 0.7	3 hr. 0.5 edema 0.7	5 hr. 0.5 0.3 0.7	5 hr. 0.5 edema 0.7	26 hr. 2.5 2.5 5	26 hr. 0.2
B. N. 7 years	Diphtheria convalescence 26 days	Scarlet fever Streptococci Tonsillitis	4 hr. 0.5 Injection failure 1.2×3	4 hr. 1.2×3 edema	24 hr. 1.0 2.0×3.5	24 hr. 0.7 2.0×3.5		
M. A. 21 years	Scarlet fever convalescence 20 days	Scarlet fever Streptococci Tonsillitis	4 hr. 0.5 faint 0.5 faint 3	4 hr.	24 hr. 4.0×4.5 3×4 5×6	24 hr. 0.5 0.5		
J. H. 6 years	Scarlet fever convalescence 7 days	Scarlet fever Streptococci Tonsillitis	4 hr. 0.5×0.7 0.3 3	4 hr.	24 hr. 0.5 0.5 2×2.5	24 hr. 0.5 0.5 0.5		
K. H.	Scarlet fever 27 days	Scarlet fever Streptococci Tonsillitis	5 hr. 2×2.5 1.5×0.5 2×2.5	5 hr. 2.5 0.2 1.5	28 hr. 3×3.5 1.5 3×4	28 hr. 1.5 1 1.5	72 and 96 hr. 1.5 1	72 and 96 hr. 1.5 0.5
H	Scarlet fever 28 days	Scarlet fever Streptococci Tonsillitis	5 hr.	5 hr. 2.5 2.2 1.0	28 hr. 1.5×2 1.5×2	28 hr. 1.0 0.2	72 and 96 hr. Slight	72 and 96 hr. 1
A	Diphtheria convalescence	Scarlet fever Streptococci Tonsillitis	5 hr.	5 hr.	28 hr. 0.5 0.5	28 hr. 0.5 0.5	72 and 96 hr.	72 and 96 hr.
Mr. M. 25 years	Scarlet fever 12 days	Scarlet fever Streptococci Tonsillitis	3 and 5 hr.	3 and 5 hr. 0.5 0.2	50 hr.	50 hr.	72 hr.	72 hr.
E. M.	Scarlet fever streptococcus mastoiditis 3 mo.	Scarlet fever Streptococci Tonsillitis	3 and 5 hr.	3 and 5 hr. 0.5	50 and 74 hr. 0.5	50 and 74 hr. 0.5	96 hr. hr.	96 hr. hr.
A. M. 2 years	Diphtheria-carrier 3 mo.	Scarlet fever Streptococci Tonsillitis	3 and 5 hr.	3 and 5 hr.	50 hr.	50 hr. 0.2 0.2 0.2		
R. S. 12 years	Scarlet fever 30 days	Scarlet fever Streptococci Tonsillitis	4 hr. 1.0 0.5	4 hr.	24 hr. 2 3 Slight	24 hr. 0.5 2.0 0.3		

when it averaged about 1 cm. in diameter. After 48 hours the induration began to disappear and by the end of a week was usually gone.

Streptococcus Antigen.—The reaction was similar to that with the scarlatinal-mucus antigen except that the average diameter of the area of reddening was about 1.5 cm. and the diameter of the induration about 0.5 cm.

Tonsillitis Antigen.—With the tonsillitis antigen more variation occurred than with either of the other antigens. The area of redness was usually larger, being about 3 cm., and the induration averaged about 0.5 cm.

The reactions in the convalescent diphtheria patients developed more slowly than those in the scarlatinal patients. The reddening was not pronounced until the day following. The period of maximal development, however, was about the same as in the scarlatinal cases. The induration did not, as a rule, last so long. The average measurements at the time of maximal development were as follows: (1) scarlatinal throat antigen, reddening 4 cm., induration 0.5 cm.; (2) streptococcus antigen, reddening 0.75 cm., induration 0.5 cm.; (3) tonsillitis antigen, reddening 0.7 cm., induration 0.5 cm.

The interpretation of the cutaneous reactions offers some difficulty, but the reaction of the skin in convalescent scarlatinal patients to all three antigens was much more marked than the reaction of the diphtheria skin. In this connection an observation made by Heim[6] is of interest. He made von Pirquet tests for tuberculosis with 3 dilutions in a child who gave positive reactions to all three dilutions. He then, in the course of the following week, gave 3 subcutaneous injections of small quantities of tuberculin, none of which produced local reactions. A month after the tuberculin tests were made, the child developed scarlet fever and at the same time severe reactions occurred at the sites of all 6 tuberculin inoculations. Aside from this general hypersusceptibility to irritation of the convalescent scarlatinal skin, the significance of the skin reactions is not clear. There was, as a rule, a greater difference between the indurations following the injection of the scarlatinal-mucus antigen in scarlatinal patients and control than between the indurations caused by either of the other two antigens in scarlatinal patients and controls. On the other hand the scarlatinal skin gave more marked reddening after the injection of tonsillitis antigen than after the injection of scarlatinal antigen and streptococcus. A specificity comparable to the cutaneous tuberculin test could not be demonstrated.

[6] Wien. med. Wchnschr., 1908, 58, p. 1831.